

Contents lists available at [SciVerse ScienceDirect](http://SciVerse.ScienceDirect.com)

Food Research International

journal homepage: [www.elsevier.com/locate/foodres](http://www.elsevier.com/locate/foodres)

# The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts [*Dipteryx alata* Vog.]

Miriam Rejane Bonilla Lemos<sup>a</sup>, Egle Machado de Almeida Siqueira<sup>b,\*</sup>,  
Sandra Fernandes Arruda<sup>c</sup>, Rui Carlos Zambiasi<sup>d</sup>

<sup>a</sup> Health Sciences Postgraduation Program, Health Sciences Faculty, Campus Universitário Darcy Ribeiro, Universidade de Brasília, Brasília, DF, POBox 70910900, Brazil

<sup>b</sup> Department of Cell Biology, Biological Sciences Institute, Campus Universitário Darcy Ribeiro, Universidade de Brasília, Brasília, DF, POBox 70910900, Brazil

<sup>c</sup> Department of Nutrition, Faculty of Health Sciences, Campus Universitário Darcy Ribeiro, Universidade de Brasília, Brasília, DF, Brazil

<sup>d</sup> Posgraduate Program of Science and Agro-industrial Technology, Laboratory of Chromatography, Faculty of Agronomia Eliseu Maciel (FAEM), Universidade Federal de Pelotas (UFPel), RS, Brazil

## ARTICLE INFO

### Article history:

Received 22 March 2012

Accepted 27 May 2012

### Keywords:

Antioxidant capacity

Phenolic compounds

Baru

Roasting

## ABSTRACT

The effect of roasting on phenolic compounds and antioxidant activities in baru nuts (*Dipteryx alata* Vog) with and without peels were investigated. Approximately 50% of the total phenolic content (Folin Ciocalteu assay) and 90% of the free radical-scavenging activity (DPPH) were present in the peel of the baru nut. Among the eight phenolics identified: p-coumaric acid, ellagic acid, caffeic acid, hydroxybenzoic, catechin, ferulic acid, epicatechin and gallic acid, the last was predominant in all nut samples with values that ranged from 224.0 to 66.7 mg/100 g. The roasting process did not reduce the total phenolic content significantly compared to the raw nut ( $p > 0.05$ ). However, roasting reduced the DPPH activity by approximately 50% in the nuts with peels. With the exception of gallic acid, the content of the other seven phenolic compounds significantly decreased after the roasting process in baru nuts with peels, suggesting that a phenolic other than gallic acid is the main compound responsible for the antioxidant potential of this nut.

© 2012 Elsevier Ltd. Open access under the [Elsevier OA license](http://www.elsevier.com/locate/elsevier/oa-license).

## 1. Introduction

Oxidative stress conditions seem to be involved in the etiology of several chronic human diseases, such as cardiovascular, diabetes, cancer, genetic, metabolic and neurodegenerative diseases. The consumption of fruits and vegetables has been positively related to a reduction of the risk of developing these chronic diseases, (Phung, Makanji, White, & Coleman, 2009) and the potential of beneficial plants has been attributed to the presence of bioactive compounds that show antioxidant properties, acting as free radical scavengers or metal chelators, reducing the reactions that produce reactive oxygen species (ROS) (Liu, Liu, & Chen, 2005; Rochfort & Panozzo, 2007; Van Horn et al., 2008). The antioxidant properties of some bioactive compounds, such as phenolics, flavonoids, estrogens and carotenoids, have been widely described in animal models or in cell culture, demonstrating animal cell protection against the action of ROS and consequently protection against chronic diseases (Barreiros, David, & David, 1996; Melo, Maciel, Lima, & Nascimento, 2008; Van Horn et al., 2008). These bioactive compounds,

which are distributed throughout all plant parts, are actually the defense system of plants in response to abiotic stress or the action of pathogens to which they are subjected.

The Cerrado, the second-largest biome of Brazil, with edaphic climate conditions similar to savannas, is distinguished by the high plant diversity. The high plant diversity and the endemism found in this ecosystem results from habitat heterogeneity and from the different soil conditions (Mendonça et al., 1998).

Baru is a native shrub from the Cerrado belonging to the *Leguminosae* *Faboideae* family, which flowers from November to May and produces fruit from July to October (Bozza, 2004; Sano, Brito, & Ribeiro, 2006). Previous studies have shown that baru nuts have high nutritional value, being a source of minerals such as iron, zinc and calcium, proteins and unsaturated fatty acids, in addition to the high level of phytic acid, tannins and tocopherols (Marin, Siqueira, & Arruda, 2009; Takemoto, Okada, Garbelotti, Tavares, & Aued-Pimentel, 2001). Recently, a study demonstrated that the consumption of the baru nut protects tissues against iron-induced oxidative stress in rats (Siqueira et al., 2012). However, like the majority of *leguminosae* seeds, baru nuts also contain a trypsin inhibitor that can be inactivated by heat treatment before human consumption to inactivate this inhibitor (Botezelli, Davide, & Malavasi, 2000; DiPietro & Liener, 1989). The aim of this study was to identify and quantify the bioactive compounds in Brazilian baru nuts and evaluate the effect of roasting on the bioactive compounds and the antioxidant capacity of the nut.

\* Corresponding author at: Laboratório de Bioquímica da Nutrição, Bloco J, 1º andar, Departamento de Biologia Celular, Instituto de Ciências Biológicas, Universidade de Brasília, Campus Universitário Darcy Ribeiro, Asa Norte, 70.910.900, Brasília, DF, POBox 70910900, Brazil. Tel.: +55 61 3107 3099; fax: +55 61 3273 3676.

E-mail addresses: [rebonilla@gmail.com](mailto:rebonilla@gmail.com) (M.R.B. Lemos), [eglemasi@gmail.com](mailto:eglemasi@gmail.com) (E.M.A. Siqueira), [arruda@unb.br](mailto:arruda@unb.br) (S.F. Arruda), [zambiasi@gmail.com](mailto:zambiasi@gmail.com) (R.C. Zambiasi).

## 2. Materials and methods

### 2.1. Chemicals

The DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) from Sigma-Aldrich (Steinheim, Germany). Phenolic standard chromatographic (hydroxycinnamic acids, caffeic acid, ferulic acid, p-coumaric acid, hydroxybenzoic acid, gallic acid, ellagic acid, p-idroxibenzóico; flavonols: quercetin, kaempferol, myricetin and flavanols (+) catechin, (–) epicatechin, all with 96–99% purity) were obtained from Sigma (St. Louis, MO). Methanol (for extraction) and Folin–Ciocalteu reagent were purchased from Vetec (Vetec fine-chemical Ltda, Brazil). Methanol HPLC grade was purchased from HPLC Vetec (Vetec fine-chemical Ltda, Brazil).

### 2.2. Plant materials

Baru nuts [*Dipteryx alata* Vog.] were obtained from local producers in Pirenópolis, Goiás, Brazil (minimum of 1 kg fresh weight of each fruit). The nuts were selected to discard chipped, decayed or broken grains. The selected nuts were homogenized and distributed randomly (Galeazzi, Lima, Colugnati, Padovani, & Rodriguez-Amaya, 2002) into two groups: raw nuts with peels and raw nuts without peels (manually removed). Half of the nuts from both groups were taken for roasting by spreading the nuts on trays that were placed into a dry oven at 150 °C for 45 min, procedure similar to the method used by the producer. After roasting, the baru nuts were ground, packed in colorless plastic bags (400 g) and stored at –80 °C until analysis.

### 2.3. Free radical scavenging assay (DPPH<sup>•</sup>)

The antioxidant capacity was determined in methanol extracts of baru using the stable radical DPPH<sup>•</sup> (2,2-diphenyl-1-picrylhydrazyl), according Brand-Williams, Cuvelier, and Berset (1995) and modified by Sánchez-Moreno, Larrauri, and Sauro-Calixto (1998). Briefly, to 5 g of each sample was added to 20 ml of methanol, homogenized and stored at –4 °C. After 24 h, the homogenate was centrifuged for 15 min. The test was carried out in tubes protected with aluminum foil containing 10 µL of methanol extract of nuts, 90 µL of methanol and 3.9 mL of DPPH<sup>•</sup>, totaling a final volume of 4.0 mL. The reaction occurred in the dark, and the values of absorbance at 517 nm after 30 min of reaction were determined using a spectrophotometer (Ultrospec 2000 UV/Visible, Pharmacia Biotech). The ability to scavenge the DPPH<sup>•</sup> radical was determined using the standard curve ( $y = 0.508x$ ,  $r^2 = 0.9975$ ), obtained with Trolox (0 to 1.5 mmol/L) and DPPH<sup>•</sup>. The results were expressed as TE, i.e., Trolox equivalent antioxidant capacity (µmol of Trolox/100 g nut dry matter).

### 2.4. Determination of total phenolic compounds

The total polyphenols were quantified using the colorimetric method of Folin–Ciocalteu (Singleton & Rossi, 1996). Briefly, 2.0 g of baru nuts was ground with 20 mL of methanol and subjected to sonication for 10 min and then filtered using filter paper. The total volume was brought to 50 mL with the addition of methanol. The assay reaction consisted of 1.0 mL of the methanol extract and 0.5 mL of Folin–Ciocalteu reagent. After 3 min, the reaction was stopped with 1.5 mL of 20% sodium carbonate and kept at room temperature for 2 h, protected from light. The absorbance was obtained at 765 nm (Ultrospect 2000, Pharmacia Biotech). The phenolic concentration was determined using a standard curve prepared with a standard solution of gallic acid, and the results were expressed as mg gallic acid/100 g of baru nuts (GAE/100 g).

### 2.5. Individual phenolic identification

Phenolic compounds were extracted from the baru nuts according to the methodology of Hakkinen, Karenlampi, Heinonen, Mykkanen, and Torronen (1998). The identification and quantification were performed on a high performance liquid chromatography system (HPLC). Briefly, to 5 g of macerated sample was added 30 mL of methanol and 4.9 mL of hydrochloric acid (final concentration of 1.2 mol/L HCl), and the total volume was brought to 50 mL with the addition of methanol. The extract was shaken in a 35 °C water bath in a dark room. After 24 h, the extract was cooled and filtered. The filtrate was dried using a rotary evaporator at 35 °C. The residue was dissolved in methanol to a final volume of 5 mL and centrifuged (7.000×g/10 min). An aliquot of the supernatant (30 µL) was injected into the liquid chromatographic system. The chromatograph system consisted of an HPLC-Shimadzu instrument with an autosampler, UV–visible detector at 280 nm, reversed phase column RP-18 CLC-ODS (5 µm, 4.6 mm×150 mm) and a guard column CLC- GDS 5 µm, 2 cm×4 mm, Supelco), both placed in an oven at 25 °C. Elution was carried out at a flow rate of 0.9 mL/min under a gradient of aqueous acetic acid (99:1 v/v, solvent A) and methanol (solvent B) from 100% A to 60% A in 25 min and maintained constant for 2 min. Then, the gradient was changed from 60 to 95% A in 10 min, maintained constant for 5 min, and finally it was changed again from 95 to 100% A until the end of the chromatography (45 min), according to Zambiasi (1997). A tentative identification and quantification of the phenolic compounds were done by using the retention time of external standards and by calibration curves obtained with the following standards: gallic acid ( $y = 3.2551e - 0.07x$ ;  $R^2: 0.9969$ ); (+)-catechin ( $y = 1.3851e - 0.06x$ ;  $R^2: 0.9951$ ); ferulic acid ( $y = 3.2716e - 0.07x$ ;  $R^2: 0.9995$ ); (–)-epicatechin ( $y = 1.3590e - 0.06x$ ;  $R^2: 0.9977$ ); ellagic acid ( $y = 5.8751e - 0.07x$ ;  $R^2: 0.9977$ ); p-coumaric acid ( $y = 2.14083e - 0.07x$ , with  $R^2: 0.9903$ ); caffeic acid ( $y = 2.9333e - 0.07x$ ;  $R^2: 0.9989$ ); p-hydroxybenzoic acid ( $y = 6.4457e - 0.07x$ ;  $R^2: 0.9955$ ); quercetin ( $y = 6.2498e - 0.07x$ ;  $R^2: 0.9926$ ), myricetin ( $y = 1.3574e - 0.06x$ ;  $R^2: 0.9997$ ) and kaempferol ( $y = 5.6644e - 0.07x$ ;  $R^2: 0.9990$ ). The results were expressed as mg phenolic compound/100 g sample.

### 2.6. Total anthocyanins

The anthocyanin determination was performed according to the Lees and Francis (1972) methodology. Briefly, to 1 g of sample was added 25 mL of ethanol acidified with hydrochloric acid to a pH of 1.0. This mixture was homogenized every 5 min during 1 h. The residue was filtered, and the supernatant volume was brought to a total of 50 mL with the addition of ethanol. The absorbance was measured at 520 nm in a spectrophotometer (model Ultrospec 2000) using ethanol to calibrate the equipment. The results were expressed as mg cyanidin 3-glycoside/100 g nut.

### 2.7. Statistical analyses

All analytical tests were performed in triplicate. Comparisons among the baru nuts from each treatment were performed by ANOVA with a Bonferroni correction using SPSS, version 17.0. Results with  $p < 0.05$  were considered significantly different.

## 3. Results and discussion

The determination of the total content of phenolic compounds in baru nuts (*D. alata*) was performed in methanol extract due to the high solubility of phenolic compounds in this medium (Kahkonen, Hopia, & Heinonen, 2001) and the results are shown in Table 1. The results show that the baru nut with peel has an average content of total phenolic compounds higher than to those found in seven out of ten nuts (pines, macadamias, Brazil nuts, cashews, nuts, hazelnuts and

peanuts) consumed in Brazil and the United States, which had total phenolic content ranging from 32 to 420 mg GAE/100 g (Kornsteiner, Karl-Heinz, & Elmadfa, 2006). The content found in baru nuts also exceeds the content reported for other popular nuts and those produced in the Center-South region of Brazil, such as pequi nuts [*Caryocar brasiliense*, Camb] and two species of pindo palm nuts [*B. capitata* e *B. eriosphata*], which had phenolic contents between 122 and 443 mg GAE/100 g, respectively (Lima, Silva, Trindade, Torres, & Mancini-Filho, 2007; Sganzerla, 2010).

In the present study, raw baru nuts with peels had a significantly higher content of total phenolic compounds in relation to baru nuts without peels (Table 1). The highest content of phenolic compounds found in baru nuts with peels corroborate several studies that have found high levels of phenolic compounds in fruit and vegetable peels and in other nuts (Chaves et al., 2010; Rochfort & Panozzo, 2007). The phenolic compounds are associated with a protector system of fruit and seeds against biotic (pathogen attack) and abiotic factors (UV, drought and salt stress), which may explain the higher content of bioactive compounds (Simões et al., 2007).

According to Xu and Chang (2008) the thermal treatment applied to foods of plant origin by heating or roasting causes evaporation of intracellular water, triggering chemical reactions that can change the lignocellulosic structure and promotes protein denaturation, which might result in a greater availability of plant phenolic compounds in the matrix. Therefore, a thermal process can affect both the nutritional and bioactive characteristics of foods (Mazzeo et al., 2011). In the present study, roasting caused a significant reduction of the phenolic content only in baru nuts without peels. These results suggest that the phenolic compounds present in baru nuts are thermolabile and that the peel might have a protective effect against heat.

Many authors have demonstrated a positive correlation between total phenolic content and the antioxidant capacity of fruits and vegetable (Li, Wang, Li, Li, & Wang, 2009). Kornsteiner et al. (2006) found a higher antioxidant activity and higher content of phenolic compounds in several nuts, such as walnuts (1,625 mg GAE/100 g and 179  $\mu\text{mol TE/g}$ ), pecans (1,284 mg GAE/100 g and 135  $\mu\text{mol TE/g}$ ), pistachios (867 mg GAE/100 g and 80  $\mu\text{mol TE/g}$ , and pinion (pine nuts) (32 mg GAE/100 g e 7  $\mu\text{mol TE/g}$ ). These results indicate a high correlation between the phenolic content and the antioxidant activity of the studied nuts. However, Wu et al. (2004) evaluating the lipophilic and hydrophilic antioxidant capacity of 100 types of foods, including the nuts studied by Kornsteiner et al., 2006, found a weak correlation between the total phenolic content and the antioxidant capacity measured by the ORAC test. In the present study, the antioxidant activity of baru nuts was determined by the ability of the samples to trap DPPH radical. This methodology has been widely used to evaluate the antioxidant activity of extracts and pure substances (Jung, Heo, & Wang, 2008). The correlation between the concentrations of total phenolic content and the antioxidant activity (AA) performed by Pearson's test showed a high and significant positive correlation ( $R=0.897$ ,  $p=0.001$ ) in all baru nuts studied, suggesting a strong involvement of phenolic compounds in the antioxidant activity measured by the DPPH methodology. The greatest antioxidant

capacity found in baru nuts with peels relative to the nut without peel (Table 1) has also been observed in other nuts. Villarreal-Lozoya, Lombardini, and Cisneroszevallos (2007) observed higher levels of phenolic compounds and tannin content in pecans with peels, which was associated with a higher antioxidant capacity measured by DPPH relative to the content in pecans without peels. In the present study, roasting did not significantly reduce the phenolic content in baru nuts with peels, but a 50% reduction in the capacity to sequester DPPH radical was observed. Contrary, a significant reduction in the phenolic content after roasting was observed in baru nuts without peels. These results seem to indicate a possible protection against phenolic compound loss in the nut by the peel.

Lima, Pereira, Abrahão, Duarte, and Paula (2010) also observed significant losses of phenolics content after roasting of cocoa, approximately 21.6% of total phenolics, and after roasting of coffee beans (18%) and in decaffeinated coffee (5%). However, the phenolic content losses and antioxidant capacity reduction might have different behaviors in relation to different media and heat processing. Studies with other foods show that even after being subjected to heat, the antioxidant capacity was unchanged (Turkmen, Saril, & Velioglu, 2005). Often, the antioxidant capacity is even enhanced due to the increased availability of phenolic compounds or by the formation of new compounds with antioxidant properties formed during the heating process, such as the melanoidins formed by the Maillard reaction. Turkmen et al. (2005) have observed an increase in the antioxidant capacity measured by the DPPH methodology in spinach and broccoli subjected to three different cooking processes (boiling water, steam and microwave), but no change in the antioxidant capacity was observed in leeks and pumpkins subjected to the same conditions. Nicoli, Anese, and Parpinel (1999) also observed in tomatoes and coffee subjected to heating that although the concentration of natural antioxidants decreased significantly as a result of the heat treatment, their antioxidant properties were preserved or even increased.

Anthocyanins are present in small amounts in baru nut compared with the levels in the reddish fruits such as strawberry, grape and blackberry, which have admittedly high contents of anthocyanins (30 to 627 mg/100 g) (Kuskoski, Asuero, Morales, & Fett, 2006; Moyer, Hummer, Finn, Frei, & Wrolstad, 2002). The consumption of these flavonoids is associated with the prevention of various diseases that involve oxidative stress, particularly cancer. Current evidence shows that the anthocyanins are poorly absorbed into the bloodstream, however, the phenolic acids and aldehydes, which are metabolites of anthocyanins produced by the intestinal flora, are better absorbed and they may exercise the protective effect against intracellular oxidative stress. A metabolite of these anthocyanins was recently identified as the acid 3-O-methyl gallic, which is likely a metabolite of malvidin. It is very probable that these metabolites of anthocyanins, the acid 3-O- and 2,4,6-metilgallic trihydroxy benzaldehyde are responsible for chemotherapeutic effects on some cancers, especially colon cancer (Forester & Waterhouse, 2010).

Studies have shown a rapid degradation of anthocyanins when food is subjected to heat treatment during food processing, with a positive correlation between the destruction of anthocyanins and increasing processing temperature. Processes that use high temperatures and shorter exposure to heat have been more efficient and are recommended to optimize the retention of these pigments (Março, Poppi, & Scarminio, 2008). In the present study roasting at 150 °C for 45 min did not influence a significant degradation of anthocyanins.

The samples of baru nuts were subjected to individual phenolic compound analysis to identify the composition of some bioactive compounds and determine the effect of treatments on their stability. A typical chromatogram of phenolic compounds present in samples of baru nut is shown in Fig. 1. Among the phenolic compounds identified by high performance liquid chromatography, gallic acid was predominant in all the samples that were analyzed, followed by catechin, ferulic acid, epicatechin and p-coumaric acid. Components in lower contents

**Table 1**

Content of total phenolics (mg gallic acid equivalent /100 g), total anthocyanins (mg cyanidin-3-glycoside /100 g) and Trolox equivalent antioxidant capacity ( $\mu\text{mol/L TE/100 g}$ ) in raw and roasted baru nuts, with and without peels.

Baru nuts	Total phenolics	Total anthocyanins	TE
Raw with peels	568.9 $\pm$ 28.7 <sup>a</sup>	1.06 $\pm$ 0.04 <sup>a</sup>	288.4 $\pm$ 1.8 <sup>a</sup>
Raw without peels	250.4 $\pm$ 8.7 <sup>b</sup>	0.62 $\pm$ 0.08 <sup>b</sup>	22.8 $\pm$ 1.5 <sup>c</sup>
Roasted with peels	531.8 $\pm$ 16.8 <sup>a</sup>	1.24 $\pm$ 0.18 <sup>a</sup>	149.1 $\pm$ 12.4 <sup>b</sup>
Roasted without peels	111.3 $\pm$ 1.8 <sup>c</sup>	1.20 $\pm$ 0.13 <sup>a</sup>	13.9 $\pm$ 0.5 <sup>c</sup>

Data are expressed as the means  $\pm$  SD. Values marked by the same letter within each column are not significantly different ( $p < 0.05$ ).

included ellagic acid, caffeic acid and, to a lesser extent, hydroxybenzoic acid (Table 2). Gallic acid, the majority compound found in baru nuts, is derived from hydroxybenzoic acid and together with ellagic acid are hydrolysable tannins that are released by acid hydrolysis (Wollgast & Anklam, 2000a). Peeled nuts, raw or roasted, showed a significantly lower content of all phenolic compounds identified, suggesting a higher concentration of these compounds in the peel of the nut.

The roasting process promoted a reduction in all individual phenolic contents. However, the effect of heat varied between the nut with and without peel. For example, the roasting process did not alter the gallic acid content in the baru nuts with peels. However, the gallic acid content decreased by more than 50% in the peeled nut. In addition, with the exception of the p-coumaric acid content that decreased significantly after heat treatment in both set of nuts (with and without peels), the contents of the other six phenolic compounds decreased significantly only in nuts with peels (Table 2). These results reinforce the suggestion that the thermolability of phenolic compounds depends not only on their structure but also on the food matrix.

The ferulic, caffeic and p-coumaric acids are present with the typical chemical structure of fatty hydroxycinnamics with a simple side chain of three carbons (C<sub>3</sub>–C<sub>6</sub>). The esters of these compounds are associated with reducing power through the donation of hydrogen from the hydroxyl group (Nystrom, Achrenius, Lampi, Moreau, & Piironen, 2007). These esters are polar compounds with low molecular weights and limited solubility in foods with a high fat content, which is the case of baru nuts, which are approximately 37% lipids. Also these phenolic acids are easily volatilized when subjected to heat for long periods. Shopova and Milkova (2000) evaluated the thermal decomposition of these compounds, and they found that ferulic acid esters are more stable than free ferulic acid in high-temperatures processes, such as roasting, boiling and frying. Ferulic acid, an aromatic acid present in the plant cell wall, was also identified in the phenolic profile of baru nuts. Normally, this acid is released during acid hydrolysis of lignocellulosic materials due to partial degradation of lignin (Luo, Brink, & Blanch, 2002). The hydroxycinnamic acid derivatives (p-coumaric, caffeic) are compounds that have an aromatic ring with a carbon chain consisting of three carbons attached to the ring and, in plants, usually appear in esterified or glycosylated form or bound to proteins (Degáspari & Waszczynskyj, 2004).

**Table 2**

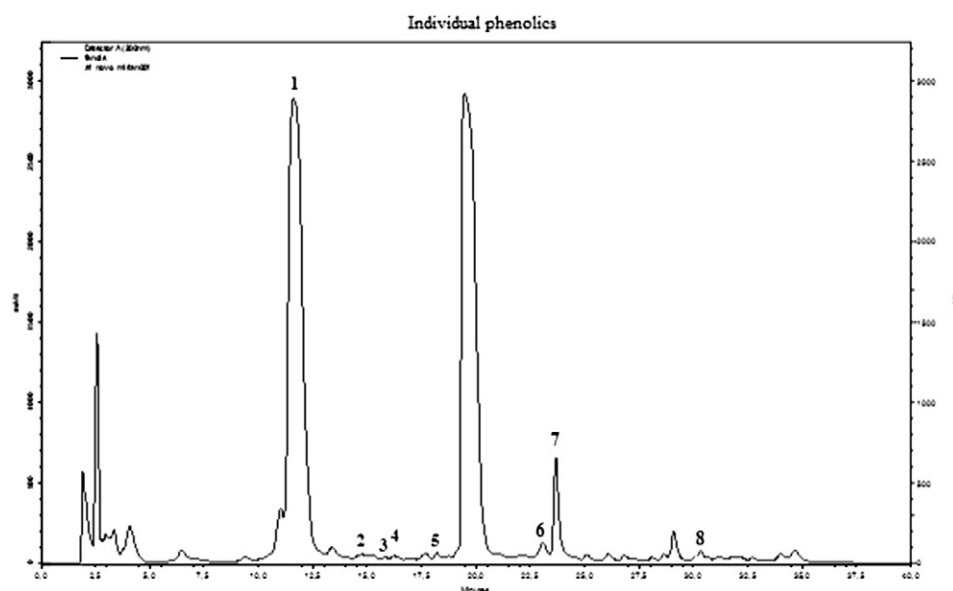
Content of phenolic compounds (mg/100 g) in baru nuts.

	Phenolic compounds in baru nut			
	Raw		Roasted	
	With peel	Peeled	With peel	Peeled
p-Coumaric acid	14.3 ± 1.1 <sup>a</sup>	3.8 ± 0.4 <sup>b</sup>	2.6 ± 0.1 <sup>b</sup>	0.3 ± 0.0 <sup>c</sup>
Ellagic acid	8.5 ± 0.9 <sup>a</sup>	4.9 ± 0.2 <sup>b</sup>	2.8 ± 0.4 <sup>c</sup>	2.0 ± 0.1 <sup>c</sup>
Caffeic acid	6.3 ± 0.9 <sup>a</sup>	2.3 ± 0.1 <sup>b</sup>	1.8 ± 0.4 <sup>b</sup>	1.1 ± 0.3 <sup>b</sup>
Gallic acid	224.0 ± 17.2 <sup>a</sup>	170.9 ± 13.8 <sup>ab</sup>	132.8 ± 20.8 <sup>b</sup>	66.7 ± 4.7 <sup>c</sup>
Hydroxybenzoic acid	2.3 ± 0.6 <sup>a</sup>	0.6 ± 0.01 <sup>b</sup>	0.3 ± 0.01 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>
Catechin	87.2 ± 3.8 <sup>a</sup>	45.6 ± 9.7 <sup>b</sup>	20.3 ± 5.2 <sup>c</sup>	13.6 ± 0.5 <sup>c</sup>
Ferulic acid	45.4 ± 4.7 <sup>a</sup>	17.5 ± 4.3 <sup>b</sup>	9.6 ± 2.8 <sup>bc</sup>	3.6 ± 0.1 <sup>c</sup>
Epicatechin	23.9 ± 3.8 <sup>a</sup>	4.8 ± 0.6 <sup>b</sup>	8.0 ± 0.7 <sup>b</sup>	2.1 ± 0.3 <sup>b</sup>

Data are expressed as the means ± SD. Means followed by the same letter in a row did not differ statistically,  $p < 0.05$ .

The catechin and ellagic acid also showed susceptibility to heat, probably caused by their highly hydroxylated structure that is vulnerable to redox reactions, causing losses when subjected to heat treatment. The (+)-catechin is a monomer that comprises the condensed tannins or proanthocyanidins, as well as (–)-epicatechin. (Efraim, Alves, & Jardim, 2011; Hii, Law, Suzannah, & Cloke, 2009; Misnawi, Jamilah, & Nazamid, 2004; Wollgast & Anklam, 2000a).

The effect of thermal processing on bioactive compounds was also investigated in vegetables (Efraim et al., 2011; Gębczyński & Lisiewska, 2006; Mazzeo et al., 2011; Pellegrini et al., 2010). Gębczyński and Lisiewska (2006) observed that cooking or boiling negatively affected the phenolic acids and flavonoids of broccoli but increased the concentration of p-coumaric acid. However, the opposite effect was observed in carrots, in which the same heat treatment increased the content of all phenolic acids, except for caffeic acid, which decreased by approximately 70%. Wollgast and Anklam (2000b) observed that, even after exposure to roasting, cocoa beans still retained polyphenols, comprised of approximately 37% catechins, 4% anthocyanins and 58% proanthocyanidins. A hypothesis that may explain the increase of the level of some phenolics compounds in plant food after heat treatment is that the heat alters the structure of some molecules, including proteins that are associated with the phenolic compounds resulting in increased levels of free phenolic compounds. This hypothesis could explain the different effects of roasting process on the content of individual phenolic compounds and the



**Fig. 1.** HPLC chromatogram at 280 nm of baru nut (roasted and peeled). Phenolic compounds: (1)Gallic acid; (2) Catechin; (3)p-Hydroxybenzoic acid; (4) Caffeic acid; (5)Epicatechin; (6)p-Coumaric acid; (7)Ferulic acid; (8) Ellagic acid.



antioxidant activity found in the present study (Efraim et al., 2011; Hii et al., 2009; Wollgast & Anklaam, 2000a; Wollgast & Anklaam, 2000b).

The roasting process reduced significantly the antioxidant capacity without change the levels of gallic acid in the baru nut with peels, while the heat treatment in the nut without peels reduced significantly the levels of gallic acid without altering the antioxidant capacity. These results suggest that other phenolic compounds might be the main bioactive compounds responsible for the antioxidant activity in this nut. The catechin and ferulic acid, for example, could be the main antioxidant compounds, once contents of these compounds decrease by approximately 50% upon roasting, which is in agreement with the reduction in the antioxidant capacity of nuts with peels after roasting. Those compounds might be responsible for the protective effect against oxidative stress observed in rats supplemented with oral iron and fed a diet containing baru nuts, as described by Siqueira et al. (2012).

#### 4. Conclusions

Baru nuts with peels showed a higher content of phenolic compounds as well as high antioxidant capacity compared to baru nuts without peels, suggesting that the peels might be a potential source of antioxidants. Although gallic acid is the predominant phenolic compound identified in the baru nut, it seems not to be the main compound responsible for the antioxidant activity. Instead, catechin, ferulic acid and epicatechin might be the main antioxidant compounds.

#### Acknowledgments

We acknowledge Fundação de Amparo à Pesquisa do distrito Federal (FAPDF) for the financial support and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship support.

#### References

- Barreiros, L. B. S., David, J. M., & David, J. P. (1996). Oxidative stress: relations between the formation of reactive species and the organism's defense. *Química Nova*, 29, 113–123.
- Botezelli, L., Davide, A. C., & Malavasi, M. M. (2000). Characteristics of fruits and seeds of four provenances of *Dipteryx alata* Vog. *Cerne*, 6, 9–18.
- Bozza, A. F. O. (2004). Aproveitamento dos frutos o cerrado. *Simpósio Ambientalista Brasileiro No Cerrado, Goiânia. Anais. SABC, Goiânia, Goiás, Brazil*.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensm-Wiss Technology*, 28, 25–30.
- Chaves, M., Citó, A. M., Lopes, J. A. D., Costa, D. A., Oliveira, C. A., Costa, A. F., et al. (2010). Total phenolics, antioxidant activity and chemical constituents from extracts of *Anacardium occidentale* L., Anacardiaceae. *Revista Brasileira de Farmacognosia*, 20, 106–112.
- Degáspari, C. H., & Waszczynski, N. (2004). Antioxidants properties of phenolic compounds. *Visão Acadêmica*, 5, 33–40.
- DiPietro, C. M., & Liener, I. E. (1989). Heat inactivation of the Kunitz and Bowman-Birk soybean protease inhibitors. *Journal of Agricultural and Food Chemistry*, 37, 39–44.
- Efraim, P., Alves, A. B., & Jardim, D. C. P. (2011). Revisão: polifenóis em cacau e derivados: teores, fatores de variação e efeitos na saúde. *Brazilian Journal of Food Technology*, 14, 181–201.
- Forester, S. C., & Waterhouse, A. L. (2010). Gut metabolites of anthocyanins, gallic acid, 3-O-methylgallic acid, and 2,4,6-trihydroxybenzaldehyde inhibit cell proliferation of Caco-2 cells. *Journal of Agricultural and Food Chemistry*, 58, 5320–5327.
- Galeazzi, M. A. M., Lima, D. M., Colugnati, F. A. B., Padovani, R. M., & Rodriguez-Amaya, D. B. (2002). Sampling plan for the Brazilian TACO project. *Journal of Food Composition and Analysis*, 15, 499–505.
- Gębczyński, P., & Lisiewska, Z. (2006). Comparison of the level of selected antioxidative compounds in frozen broccoli produced using traditional and modified methods. *Innovative Food Science and Emerging Technologies*, 7, 239–245.
- Hakkinen, S. H., Karenlampi, S. O., Heinonen, M., Mykkanen, H. M., & Torronen, A. R. (1998). HPLC method for screening of flavonoids and phenolic acids in berries. *Journal of the Science of Food and Agriculture*, 77, 543–551.
- Hii, C. L., Law, C. L., Suzannah, S., & Cloke, M. (2009). Polyphenols in cocoa (*Theobroma cacao* L.). *As. Asian Journal of Food and Agro-Industry*, 2, 702–722.
- Jung, M. J., Heo, S. -I., & Wang, M. -H. (2008). Free radical scavenging and total phenolic contents from methanolic extracts of *Ulmus davidiana*. *Food Chemistry*, 108, 482–487.
- Kahkonen, M. P., Hopia, A. I., & Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry*, 49, 4076–4082.
- Kornsteiner, M., Karl-Heinz, W., & Elmadfa, I. (2006). Tocopherols and total phenolics in 10 different nut types. *Food Chemistry*, 98, 381–387.
- Kuskoski, E. M., Asuero, A. G., Morales, M. T., & Fett, R. (2006). Wild fruits and pulps of frozen fruits: antioxidant activity, polyphenols and anthocyanins. *Ciência Rural*, 36, 1283–1287.
- Lees, D. H., & Francis, F. J. (1972). Standardization of pigment analysis in cranberries. *Hortscience*, 7(1), 83–84.
- Li, H., Wang, X., Li, Y., Li, P., & Wang, H. (2009). Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chemistry*, 112, 454–460.
- Lima, A. R., Pereira, A. F. P., Abrahão, A. S., Duarte, S. M., & Paula, F. B. A. (2010). Coffee bioactive compounds: *in vitro* antioxidant activity of green and roasted coffees before and after decaffeination. *Química Nova*, 33, 20–24.
- Lima, A., Silva, A. O., Trindade, R. A. T., Torres, R. P. T., & Mancini-Filho, J. (2007). Composição química e compostos bioativos presentes na polpa e na amêndoa do pequi (*Caryocar brasiliense*, Camb.). *Revista Brasileira de Fruticultura*, 29(3), 695–698.
- Liu, R. H., Liu, J., & Chen, B. (2005). Apples prevent mammary tumors in rats. *Journal of Agricultural and Food Chemistry*, 53, 2341–2343.
- Luo, C., Brink, D. L., & Blanch, H. W. (2002). Identification of potential fermentation inhibitors in conversion of poplar hydrolyzate to ethanol. *Biomass and Bioenergy*, 22, 125–138.
- Março, P. H., Poppi, R. J., & Scarminio, I. S. (2008). Analytical procedures for identifying anthocyanins in natural extracts. *Química Nova*, 31, 1218–1223.
- Marin, A. M., Siqueira, E. M. A., & Arruda, S. F. (2009). Minerals, phytic acid and tannin contents of 18 fruits from the Brazilian savanna. *International Journal of Food Science*, 60, 180–190.
- Mazzeo, T., N'Dri, D., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrine, N. (2011). Effect of two cooking procedures on phytochemical compounds, total antioxidant capacity and color of selected frozen vegetables. *Food Chemistry*, 128, 627–633.
- Melo, E. A., Maciel, M. I. S., Lima, V. L. A., & Nascimento, R. J. (2008). Capacidade antioxidante de frutas. *Brazilian Journal of Pharmaceutical Sciences*, 44, 193–201.
- Mendonça, R. C., Felfili, J. M., Walter, B. M. T., Silva Júnior, M. C., Rezende, A. V., Filgueiras, T. S., & Nogueira, P. E. (1998). Flora vascular do Cerrado. In S. M. Sano, & S. P. Almeida (Eds.), *Cerrado: Ambiente e Flora* (pp. 286–556). Brasil: Empresa Brasileira de Pesquisa Agropecuária do Brasil (EMBRAPA-CPAC).
- Misnawi, J. S., Jamilah, B., & Nazamid, S. (2004). Effect of polyphenol concentration on pyrazine formation during cocoa liquor roasting. *Food Chemistry*, 85, 73–80.
- Moyer, R. A., Hummer, K. E., Finn, C. E., Frei, B., & Wrolstad, R. E. (2002). Anthocyanins, phenolics, and antioxidants capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. *Journal of Agricultural and Food Chemistry*, 50, 519–525.
- Nicoli, M. C., Anese, M., & Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science & Technology*, 10, 94–100.
- Nystrom, L., Achrenius, T., Lampi, A. -M., Moreau, R. A., & Piironen, V. (2007). A comparison of the antioxidant properties of steryl ferulates with tocopherol at high temperatures. *Food Chemistry*, 101, 947–954.
- Pellegrini, N., Chiavaro, E., Gardana, C., Mazzeo, T., Contino, D., & Gallo, M. (2010). Effect of different cooking methods on color, phytochemical concentration, and antioxidant capacity of raw and frozen Brassica vegetables. *Journal of Agricultural and Food Chemistry*, 58, 4310–4321.
- Phung, O. J., Maknani, S. S., White, C. M., & Coleman, C. I. (2009). Almonds have a neutral effect on serum lipid profiles: a meta-analysis of randomized trials. *Journal of the American Dietetic Association*, 109, 865–873.
- Rochfort, S., & Panozzo, J. (2007). Phytochemicals for health, the role of pulses. *Journal of Agricultural and Food Chemistry*, 55, 7981–7994.
- Sánchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, A. (1998). Procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, 76, 270–276.
- Sano S. M., Brito M. A. de, & Ribeiro, J. F. (2006). Baru. In: *Frutas nativas da região Centro-Oeste do Brasil*. 1st Ed.; Vieira, R. F.; Costa, T. da S. A.; Silva, D. B. da; Ferreira, F. R.; Sano, S. M., (Ed.) Embrapa Recursos Genéticos e Biotecnológicos. Brasília (pp. 76–98). Brasil: Empresa Brasileira de Pesquisa Agropecuária do Brasil (EMBRAPA-CPAC).
- Sganzerla, M. (2010). Caracterização físico-química e capacidade antioxidante do butiá. – PPGCTA. Universidade Federal de Pelotas, Pelotas-RS, Dissertação (Mestrado), 107.
- Shopova, N., & Milkova, T. (2000). Thermal decomposition of cholesteryl esters of cinnamic acid derivatives and their effect on the alphetetralylhydroperoxide free-radical-induced decomposition. *Thermochimica Acta*, 356, 101–107.
- Simões, C. M. O., Schenkel, E. P., Gosmann, G., Mello, J. C. P., Mentz, L. A., & Petrovick, P. R. (2007). *Farmacognosia: da planta ao medicamento* (6th ed.). Florianópolis: Publisher Federal University of Santa Catarina – Brasil.
- Singleton, V. L., & Rossi, J. A. (1996). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.
- Siqueira, E. M. A., Marin, A. M. F., Arruda, S. F., Cunha, M. S. B., Fustinoni, A. M., & Dourado, L. P. (2012). Consumption of baru seeds [*Dipteryx alata* Vog.], a Brazilian savanna nut, prevents iron-induced oxidative stress in rats. *Food Research International*, 45, 427–433.
- Takemoto, E., Okada, I. A., Garbelotti, M. L., Tavares, M., & Aued-Pimentel, S. (2001). Composição química da semente e do óleo de baru (*Dipteryx alata* Vog.) nativo do Município de Pirenópolis, Estado de Goiás. *Revista do Instituto Adolfo Lutz*, 60, 113–117.
- Turkmen, N., Saril, F., & Velioglu, Y. S. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chemistry*, 93, 713–718.

- Van Horn, L., Mc Coin, M., Kris-Etherton, P., Burke, F., Carson, J., & Champagne, C. (2008). The evidence for dietary prevention and treatment of cardiovascular disease. *Journal of the American Dietetic Association*, 108, 287–331.
- Villarreal-Lozoya, J. E., Lombardini, L., & Cisneroszevallos, L. (2007). Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoensis* (Wangenh.) K. Koch] cultivars. *Food Chemistry*, 102, 1241–1249.
- Wollgast, J., & Anklam, E. (2000a). Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International*, 33, 423–447.
- Wollgast, J., & Anklam, E. (2000b). Polyphenols in chocolate: is there a contribution to human health? *Food Research International*, 33, 449–459.
- Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry*, 52, 4026–4037.
- Xu, B., & Chang, S. K. C. (2008). Total phenolics, phenolic acids, isoflavones, and antioxidant properties of yellow and black soybeans as affected by thermal processing. *Journal of Agricultural and Food Chemistry*, 56, 7165–7175.
- Zambiasi, R. C. (1997). *The role of endogenous lipid components on vegetable oil stability*. These in Foods and Nutritional at the Sciences Interdepartmental Program. Manitoba, Canada: University of Manitoba Winnipeg [<http://hdl.handle.net/1993/931>]